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Novel electrochemical sensor for lab-on-a-chip and biomedical technology

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Introduction:

Culturing of organotypic brain tissues is a routine procedure in neural research. The visual inspection of the medium is the only way of determining the state of the tissue. At the end of culturing, post-processing techniques such as HPLC can be used to measure the concentration of the secreted metabolites in the waste products. Continuous measurements would enable improved monitoring as compared to the end-point assay. Here, we developed a sensor system capable of real time measurements of the analytes directly secreted from the tissue. The presented system can be readily integrated in the standard procedures allowing for better assessment of the progress of the culturing.

The sensor system was initially developed for monitoring of cells and tissue cultures but has lately been considered for, and tested in, a wide range of applications. Some of these include pathogen detection and integration in microfluidic devices for sample preparation.

Fabrication

The membrane electrodes can be fabricated in several different ways, depending on material choices and available equipment. Currently sputtering have been used for creating electrodes on membrane inserts as the one seen in Figure 1. The increased temperatures encountered during sputtering process has been seen to have some effect on the membrane morphology but not in a destructive manner.

For fabrication of electrodes on flat membranes E-beam evaporation has been used with great success. The process work at room temperature and thus does not change the morphology of the membrane material. An example of a membrane fabricated with E-beam evaporation can be seen in Figure 2.

The technique to fabrication the electrode pattern is based on the use of shadow masks as illustrated in Figure 3. In the example the membranes (white) are clamped between to layers of polymethylmethacrylate (PMMA). The bottom layer serves as an alignment for the membranes and the top is the shadow mask with the desired electrode pattern.

Once the membranes are placed securely between the PMMA sheets in a holder they are placed in the E-beam evaporator and the metal of choice is deposited in the desired thickness.

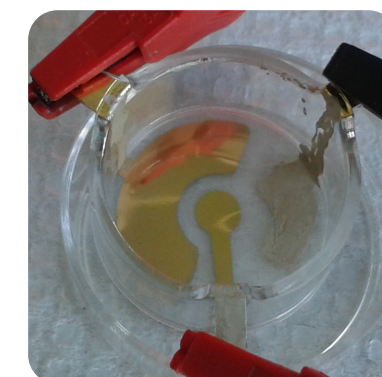


Figure 1. Membrane electrodes fabricated on a cell culture insert using sputtering. The electrodes have been fabricated in gold and silver respectively.



Figure 2. Gold electrodes fabricated on a PC membrane using E-beam evaporation.

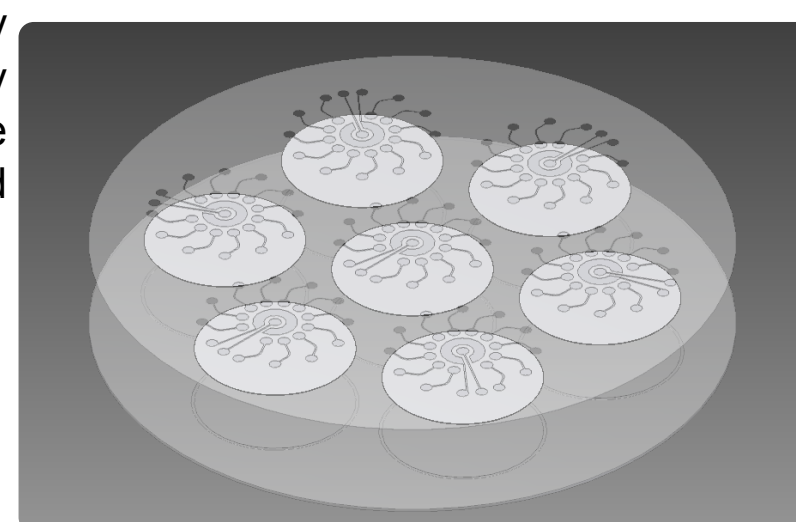


Figure 3. Arrangement of shadowmask, membranes and alignment mask for fabrication of electrodes on flat membranes using E-beam evaporation.

Characterisation

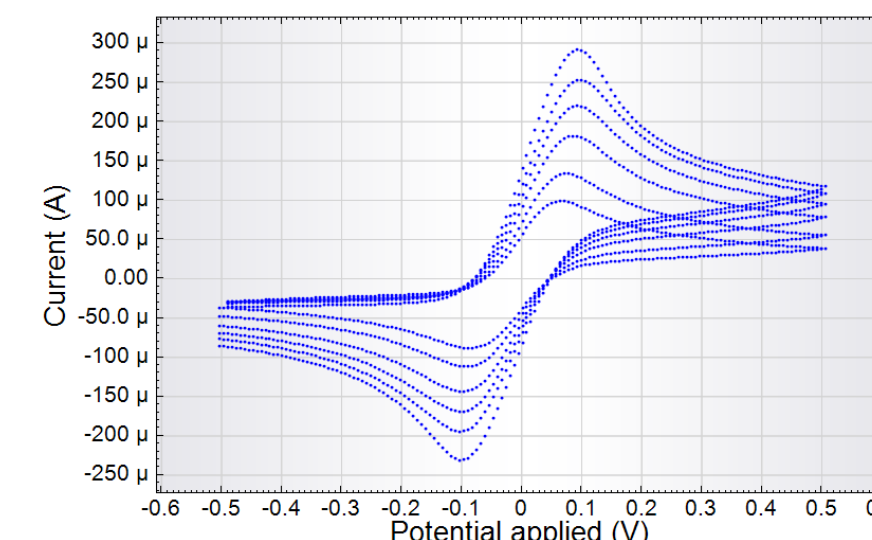


Figure 4. Representative CV performed in 10 mM ferri ferrocyanide in PBS. The CVs were performed at scan rates of 500 mV/s, 400 mV/s, 300 mV/s, 200 mV/s, 100 mV/s and 50 mV/s in the listed order. Identical measurements have been performed on numerous electrodes on a number of different membranes with similar results. A cleaning procedure was tested but was seen to have no positive effect.

Long term stability

In order to test the stability of the electrodes during storage CVs in 10 mM ferri ferrocyanide were performed every second week on three different electrodes. Three of the measurements on one of the electrodes can be seen in Figure 5. The graphs were obtained in the following order: red, green and blue. As can be seen the signal did not change considerably.

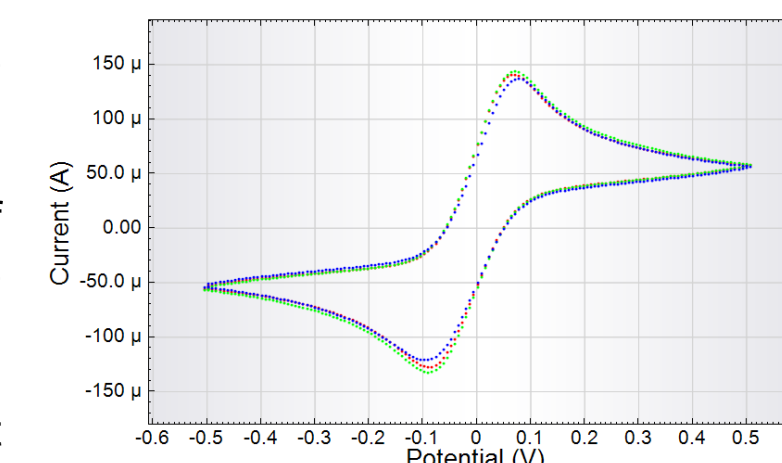


Figure 5. CV performed in 10 mM ferri ferrocyanide in PBS on a single electrode. The three CVs were performed 2 weeks apart.

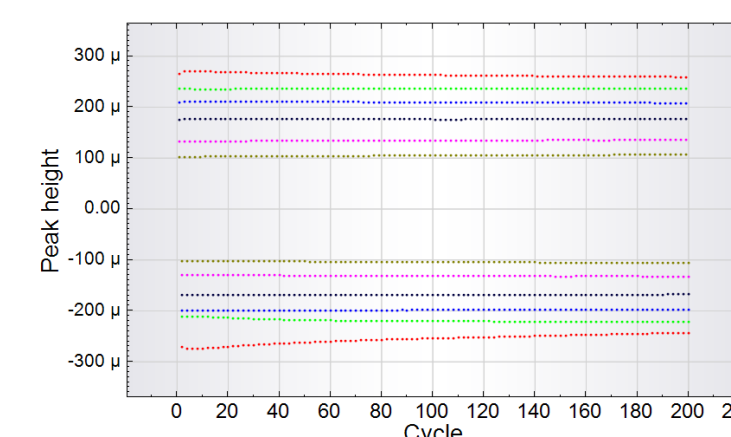


Figure 6. Peak potential of 100 continuous cycles performed in 10 mM ferri ferrocyanide at 500 mV/s, 400 mV/s, 300 mV/s, 200 mV/s, 100 mV/s and 50 mV/s. The electrodes appear to be stable during long term measurements.

Flow through the membrane

In order to determine if the deposition of the electrodes affects the flow through the membranes to tests were performed. First the membranes were studied using SEM. One of the resulting pictures can be seen in Figure 7. Based on these observations the pore size was unchanged after metal deposition.

The second test performed was a flow test where the flow through coated and uncoated membranes were measured. For membranes with a pore size of 0.45 μm (smallest tested) the flow rate was found to be 1.94 for uncoated membranes and 1.67 for coated membranes.

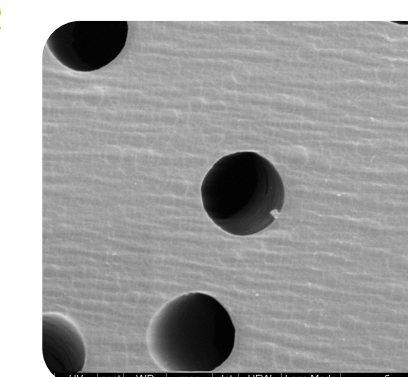


Figure 7. SEM picture of PC membrane with 200 nm gold. The pore size is unchanged.

Integration

As the membrane electrodes are intended for use in e.g. microfluidic devices it was tested if they could be easily integrated. For one use devices thermal bonding was tested. It was found that the bonding procedure (heat and pressure) did not damage the electrodes and that the signal was comparable before and after bonding. For reusable devices clamping using an o-ring and screws were tested. It was found that the signal did not change during clamping and that the membrane electrodes could easily be removed as long as they were not allowed to dry out in the device (a few electrodes broke when the dry membrane was removed).



Figure 8. Device for testing membrane electrodes. The membrane holder was designed in two versions, one for bonding and one for clamping. Both were fitted with a custom PCB board for connection to a potentiostat.

Proof of concept:

As the membrane electrodes were initially developed for measuring on cell cultures and tissue it was decided to do dopamine sensing as a preliminary test. The result of the first measurement can be seen in Figure 9. The graph shows an amperometric measurement performed in PBS purged with nitrogen and dopamine was added every 100 s after 300 s. The baseline was adjusted in Nova 1.10. The measurement was made as a preliminary proof of concept and no optimization has been made.

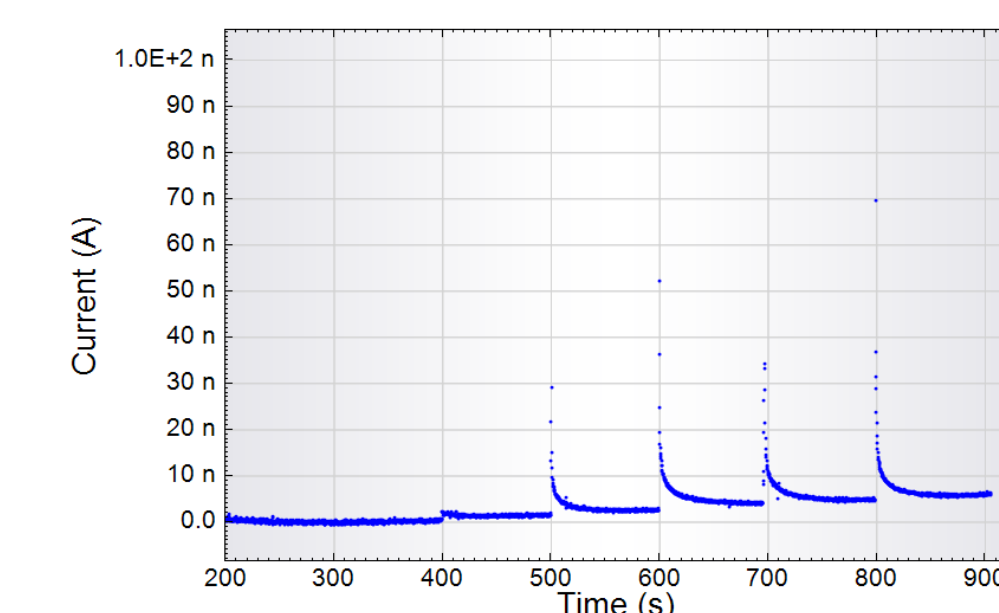


Figure 9. Amperometric measurement of addition of dopamine to a nitrogen purged PBS solution. Dopamine was added every 100 s from 300 s.

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